Possible links between holothurian lipid compositions and differences in organic matter (OM) supply at the western Pacific abyssal plains

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Abstract

Deep-sea benthic communities depend on the export of organic matter (OM) from the surface ocean. However, the effects of the pelagic-benthic coupling and the specific link between changing seasonal OM inputs and physiological changes of the mega-benthic community remain unclear. In this study, we identified differences in OM quality and quantity at two abyssal seafloor sites in the western Pacific Ocean and noted possible links between overlying primary production and the lipid composition of several deep-sea holothurian species. Phytopigment concentrations of the surface sediment were up to 16-times greater at the high productivity area (39ºN) than at the oligotrophic area (1ºN). Total carbohydrate and protein concentrations were also significantly higher at 39ºN than 1ºN, although to a lesser extent than for phytopigments. Holothurian abundances were almost 40 times higher at 39ºN than 1º N. Significant differences were detected in the fatty acid (FA) compositions of the holothurian tissues in terms of proportions of the main food source indices (phytoplankton, zooplankton and bacterial FA), suggesting different food sources in the two areas. Phytodetritus and bacteria were the most dominant dietary sources at 39ºN and 1ºN, respectively. Stable carbon and nitrogen isotopic compositions did not contradict the FA data indicating that holothurians fed on both phytodetritus and bacteria from the sediments.

Overall, our results show that high densities of abyssal holothurians at 39ºN is linked with the high quality of the sedimentary OM associated with the net primary production at the surface. Further, the differences in phytodetritus inputs may lead to a different lipid composition as a consequence of different feeding habits, although there may be some other mechanisms behind. This study provides fundamental knowledge on lipid compositions of abyssal holothurians in relation to oceanic settings, thus improves our understanding of the ecosystem functioning in abyssal plains.

1. Introduction

The deep-sea floor has one of the highest levels of biodiversity on Earth and its maintenance is essential to ecosystem stability (Loreau & Mazancourt, 2013; Tilman et al., 2014). Pelagic and benthic communities in almost all deep-sea habitats feed on organic matter (OM) sinking through the water column from the euphotic zone (Smith et al., 2008; Danovaro et al., 2014). Although most OM is consumed in the water column before reaching deep-sea sediments, the vast area of the ocean floor means that the deep sea is of global importance for carbon, nitrogen and phosphorus cycling (Dell'Anno et al., 2005). Thus, variations in food supply to the seafloor in space and time are major drivers of change in deep-sea ecosystems and subsequent biogeochemical cycles (Ruhl and Smith, 2004). It has been shown from long-term deep-sea sediment traps and benthic camera studies in the Pacific and Atlantic Oceans that decadal-scale climatic change can modify the ecosystem structure and function of deep-sea communities through changes in particulate organic carbon (POC) fluxes to the seafloor (Ruhl and Smith, 2004, Smith et al., 2009), altering patterns of diversity and ecosystem functioning. However, the relative importance of food supply, and whether all taxa respond in the same way, has been difficult to determine, because environmental drivers change at different rates across regions and oceans. Understanding the way this detrital food resource is allocated to different physiological functions within a species, may explain why seasonal fluxes play a crucial role in the structure of the benthic community *via* benthic-pelagic coupling.

Holothurians are megafaunal organisms that play a key role in most abyssal soft sedimentary environments, dominating megafaunal abundance and biomass (Sibuet et al., 1982; Billett, 1991; Roberts et al., 2000, Amaro et al., 2010, 2015). They are major consumers of phytodetrital OM being responsible in rapidly depleting OM in abyssal sediments (Bett et al., 2001; de Leo, et al., 2010). Through deposit feeding and sediment reworking, they mostly affect the availability and composition of food to other benthic organisms (Smallwood et al. 1999, Ginger et al. 2001, Witbaard et al. 2001, McClain and Barry, 2010), which can lead to major impacts into the ecosystem (Huffard et al., 2016). Shifts in abyssal holothurian populations have been related to changes in phytoplankton assemblages at the surface and with the quantity and quality of POC fluxes to the seafloor (Wigham et al., 2003; Ruhl and

Smith, 2004; Smith et al., 2006, 2008; Billett et al., 2010, Wolff et al., 2011, Amaro et al., 2015). According to Smith et al., (2008), such changes will then affect the structure and function of deep-sea ecosystems, making them potential indicators of climate change of the deep sea and carbon remineralization processes (Glover et al., 2010). It is therefore important to know how abyssal holothurian feeding habits differs between species and POC fluxes.

Lipids are useful biomarker tools in understanding food sources of deep-sea organisms (Ginger et al. 2001). They are key biochemical components, being functionally involved in energy storage (fatty acids-FA as triacylglycerides; TAG) and cell membrane components (FA as phospholipids, sterols), as well as in hormonal regulation (steroids). FA are particularly useful biomarkers for identification of macro- and microplankton species and their contribution to animal diets (Sargent et al. 1987, Virtue et al. 2000, Ginger et al., 2001, Neto et al., 2006, Drazen et al., 2008, Jeffreys et al., 2009, Parzanini et al., 2018). The method has been widely used and consequently there is a large database of lipid components taken from pure strains of many marine unicellular organisms including phytoplankton and zooplankton (e.g. Sargent et al. 1987, Parrish et al., 2000, Parzanini et al., 2018). By investigating lipid compositions of abyssal holothurians, we can understand their possible food sources (i.e., OM from oceanic surface, zooplankton which consume phytoplankton, or bacteria inhabiting in sediments), thus allowing detailed understandings on the importance of holothurians in the ecosystem functioning, as surface-deposit feeders.

The present study was carried out at two abyssal stations in the western Pacific to test whether there is a potential link between the lipid biochemistry of deep-sea holothurians and the differences in OM supply, influencing holothurian nutritional ecology and, consequently, ecosystem functioning. For that, we investigated several abyssal holothurians species from 2 abyssal sites to determine their fatty acid compositions and we measured the biochemical composition (total organic carbon, total nitrogen and their isotopic composition) and the quality of the OM in the sediment (in terms of proteins, carbohydrates and lipids). Furthermore, to complement our lipid analyses, we estimated their trophic status by means of stable carbon and nitrogen isotopes at the two abyssal stations.

2. Material and Methods

151 *2.1 Study sites*

152 Sediment and holothurian samples were collected from two abyssal stations of
153 the western Pacific Ocean with varying OM fluxes. The site 1ºN is located northeast
154 from the Ontong Java Plateau, while 39ºN is located at the seaward side of the Japan
155 Trench (Figures 1 and 2). There were no apparent topographical depression or hills
156 around the study sites. The two areas have differences in surface primary production
157 based on the satellite images
158 (https://neo.sci.gsfc.nasa.gov/view.php?datasetId=MY1DMM_CHLORA); annual net
159 primary production at 1ºN and 39ºN in each sampling year (2013 for 1ºN and 2014
160 for 39ºN: see details for section 2.2. and 2.3.) was 106 g C m^{-2} $year^{-1}$ and 324 g C m^{-2}
161 $year^{-1}$, respectively. The POC fluxes to the seafloor were calculated as 485 mg C m^{-2}
162 y^{-1} and 1086 mg C m^{-2} yr^{-1} at 1ºN and 39ºN, respectively, based on seasonality in
163 primary production and water depth (Lutz et al., 2007). Net primary production (NPP)
164 showed a strong seasonality at 39ºN in comparison to 1ºN. The NPP at 39ºN ranged
165 from 278 mg C m^{-2} d^{-1} in January to 2585 mg C m^{-2} d^{-1} in April, while those in 1ºN
166 ranged 175 mg C m^{-2} d^{-1} in November to 412 mg C m^{-2} d^{-1} in April (Behrenfeld and
167 Falkowski, 1997,
168 http://www.science.oregonstate.edu/ocean.productivity/index.php). Based on the
169 observation of sediment cores collected during the cruises, surface sediments of 1ºN
170 consisted of red clay and planktonic foraminiferal tests, whereas at 39ºN consisted of
171 diatomaceous ooze (Nomaki, H. unpublished data).
172
173 *2.2 Seafloor observations and megafauna quantification*

174 Megafaunal abundances were estimated using the video images collected
175 during R/V Yokosuka YK13-09 and YK13-12 cruises in September and November
176 2013 (1ºN), and the YK14-12 cruise in July 2014 (39ºN). In total, five independent
177 transects were made using the manned submersible *Shinkai 6500* (Table 1). Due to
178 sampling logistics other than video surveys, the surveyed area during dive#1395 at
179 39ºN was very limited, leading to a narrower observation area than 1ºN. The surface
180 area of the seafloor images observed by the fixed camera of *Shinkai 6500* was
181 calculated following the method described by Nakajima et al. (2014). In brief, the
182 camera view angle and camera tilt were fixed for all images and the altitude and tilt of
183 the *Shinkai 6500* were obtained from dive metadata and calculated for each 10-second
184 period. Images with poor resolution and not suitable for identification were discarded

(Nakajima et al. 2014). Total megafauna and holothurians were counted and their density (ind. ha^{-2}) was determined by dividing the total number of individuals counted on each transect by the total transect area annotated for that sampling period.

2.3. Holothurians and sediment sampling

Four holothurian species observed in video images, namely *Deima validum*, *Psychropotes longicauda*, *Pseudostichopus trachus* and *Scotoplanes globosa* were collected using a suction sampler attached to the *Shinkai 6500* submersible (Table 2). *Psychropotes longicauda* was collected from both 1ºN and 39ºN while the other species were only collected from a single site, because they only occurred at either 1ºN or 39ºN (see section 3.1 for more detail).

All organisms were returned to the ship inside the bio-box attached to the sample basket or in the rotated containers connected to the suction sampler. Only intact animals were selected for the lipid and isotope studies. Once the submersible was back on board, the specimens were placed immediately in a temperature-controlled laboratory (4ºC). Each specimen was dissected in a sterilized petri dish using sterilized spatulas. After dissection, body wall samples were stored in clean, aluminum foil-wrapped, pre-weighed petri dishes at -80ºC. In the laboratory samples were freeze-dried (-60ºC; 10^{-2}T; 24 h) and then frozen in liquid nitrogen and ground to a coarse powder with a pestle and mortar, and finally stored (-20ºC) prior to analysis.

Undisturbed sediment samples were collected with push cores (n = 3 at each station) fitted with 82 mm inner diameter core tubes. Upon recovery, all cores were sliced at 1 cm depth intervals down to 5 cm depth and frozen at –80ºC until analysis. Only the surface 1 cm of sediments were analysed for biochemical compositions in this study.

2.4 Lipid Analysis

Methods for analysis of lipids have been described in detail elsewhere (Kiriakoulakis et al. 2001, Neto et al., 2006, Jeffreys et al., 2009). Briefly, separate aliquots of freeze-dried holothurian tissue material (0.5-1 g) were spiked with a known amount of the internal standard (5□(H)-cholestane), extracted by sonication (3 x 15 min; dichloromethane:methanol 9:1) and methylated (methanolic acetyl chloride; Christie 1982). Gas chromatography-mass spectrometry (GCMS) analyses were

219 carried out on the silylated (bis-trimethylsilyltrifluoroacetamide; BSFTA, 1 % TMS;
220 30-50 µL; 40°C; 0.5-1 h), methylated total extracts using a Trace 2000 Series gas
221 chromatograph (on-column injector; fused silica high temperature column, 60 m ×
222 0.25 mm i.d.; 0.1 µm film thickness, 5 % phenyl/95 % methyl polysiloxane equivalent
223 phase, DB5-HT, J&W; carrier gas helium at 1.6 mL min^{-1}), coupled with a
224 Thermoquest Finnigan TSQ7000 mass spectrometer (ionisation potential 70 eV;
225 source temperature 215°C; trap current 300 µA). All analyses were processed using
226 Xcalibur software. Identification of lipid compounds were performed by the
227 comparison of the retention times and mass fragmentation patterns of known lipid
228 compounds. Quantitative data were calculated by comparison of peak areas of the
229 internal standard with those of the compounds of interest, using the total ion current
230 (TIC) chromatogram. The relative response factors of the analytes were determined
231 individually for 36 representative FA, sterols and alkenones using authentic standards.
232 Response factors for analytes where standards were unavailable were assumed to be
233 identical to those of available compounds of the same class (Kiriakoulakis et al. 2004,
234 Neto et al., 2006).
235 For the interpretations and statistical analyses, individual lipids were grouped
236 into principal classes, i.e. mono-unsaturated FA (MUFAs), polyunsaturated FA
237 (PUFAs), saturated fatty acid methyl esters (Sat._FAMEs), sterols and alcohols.
238 Lipid indices for potential food sources were calculated following
239 Kiriakoulakis et al. (2011) as such, phytoplankton FA being the sum of $C_{22:6}$, $C_{20:5}$ per
240 total lipids (Harwood & Russel, 1984; Bergé & Barnathan, 2005; Duineveld et al.,
241 2012, zooplankton FA is the sum of $C_{20:1}$, $C_{22:1}$, $C_{24:1}$ per total lipids (Dalsgaard et al.,
242 2003, Kiriakoulakis et al., 2004, Bergé & Barnathan, 2005) and bacterial FA is the
243 sum of odd-numbered saturated and branched FA (Meziane & Tsuchiya 2002,
244 Dalsgaard et al., 2003).
245
246 *2.5. Total organic carbon, total nitrogen concentrations and their isotopic*
247 *compositions*
248 The holothurians and sediment samples used for total organic carbon (TOC)
249 and total nitrogen (TN) concentrations, and their carbon and nitrogen isotopic
250 compositions ($\delta^{13}C$, $\delta^{15}N$) were weighed into pre-cleaned silver capsules (Ogawa et
251 al. 2010). The samples were decalcified with 2 M HCl followed by drying on a hot
252 plate (60ºC). Dried silver capsules containing decalcified samples were sealed into

pre-cleaned tin capsules prior to isotopic analysis. Carbon and nitrogen isotopic composition along with TOC and TN content were analysed using an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific, USA) coupled to an isotope ratio mass spectrometer (Delta plus Advantage, Thermo Fisher Scientific, USA) via a ConFlo IV interface (Thermo Fisher Scientific, USA). The standard deviations of 48 analyses of L-glutamic acid standards (USGS40 and USGS41, U. S. Geological Survery, USA) were 0.09 ‰ and 0.18 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

2.6. Biochemical composition of the sedimentary OM

Protein, carbohydrate and lipid contents of the surface 1 cm sediments were determined spectrophotometrically, and concentrations were calculated from calibration curves of serum albumin, D-glucose and tripalmitine equivalents, respectively, and normalised to sediment dry weight (Danovaro, 2010). For each compound, blanks were obtained using pre-combusted sediments (450ºC for 4 h). All analyses were performed on three replicates, with approximately 0.2–1 g of wet sediment per sample. Biopolymeric carbon was defined as the sum of the carbon equivalents of total carbohydrates, proteins, and lipids (using conversion factors of 0.40, 0.49, and 0.75, respectively) and is reported as the fraction of total organic C potentially available to benthic consumers (Pusceddu et al., 2009; Danovaro et al., 2014). The concentrations of sedimentary chlorophyll-*a* and phaeopigments were determined spectrophotometrically or spectrofluorometrically, according to standard protocols (Danovaro, 2010), and their sum referred to as total phytopigment concentrations.

2.7 Statistics

Differences between deep-sea holothurians in tissue samples from the 2 regions with respect to lipid classes were tested by multivariate analysis of similarities (ANOSIM) using PRIMER 6+ software. Differences between deep-sea holothurians from the 2 regions with respect to lipid concentrations as well as diagnostic indices were investigated by a distance-based permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001; McArdle and Anderson, 2001). PERMANOVA was carried out using the PERMANOVA package included in the Primer 6+ software. These analyses were based on Euclidean distances of normalized data using 4999 random permutations of the appropriate units and with fourth root-transformed values

(Anderson and Ter Braak, 2003). The contribution of variables (lipid index) to the total dissimilarity between regions and similarity in each region was determined using SIMPER.

The measurements from the different sediment and tissues of the holothurians between the regions were most likely dependent on one another, thus hampering the application of parametric ANOVA tests, differences in C:N ratios of the sediments and in the tissues of the holothurians between the regions were separately investigated by means of non-parametric Kruskal-Wallis analyses of variance. All statistical tests were conducted using SPSS 21.0 software.

3. Results

3.1 Sedimentary OM quantity and quality

Although OM quantity (TOC and TN concentrations) of the surface sediments did not show significant differences ($p < 0.005$) between 1ºN and 39ºN (Table 3), there are large differences in OM quality between the two areas, which is indicated by both C/N ratios of sedimentary OM and in concentrations of biopolymeric compounds (lipids, proteins, and carbohydrates) (Figure 3). Phytopigment concentrations ranged from 0.005 ±0.001 mg g^{-1} at 1ºN to 0.0473 ±0.010 mg g^{-1} at 39ºN, whereas protein concentrations ranged 0.8 ± 0.2 mg g^{-1} at 1ºN to 2.7 ± 0.3 mg g^{-1} at 39ºN (Figure 3A). Total carbohydrate ranged from 2.06 ± 0.2 mg g^{-1} at 1ºN to 4.36 ± 0.6 mg g^{-1} at 39ºN and lipid concentrations ranged from 0.2± 0.1 mg g^{-1} at 1ºN to 0.7 ± 0.1 mg g^{-1} at 39ºN (Figure 3A). The contribution of carbohydrates to the total biopolymeric C was higher at 1ºN (61.3 ± 3.7 %) than at 39ºN (48.6 ± 0.1 %), while that of proteins and lipids was higher at 39ºN (36.5 ± 0.6 % for proteins and 14.9 ± 0.5 % for lipids) than at 1ºN (27.9 ± 1.8 % for proteins and 10.9 ± 1.9 % for lipids) (Figure 3B).

3.1 Video surveys

Megafaunal density was ~40 times higher at 39ºN than 1ºN (an average of 54.5 ± 27.4 ind. per ha in 1ºN and 2144.4 ± 593.4 ind. per ha in 39ºN; Table 4). For both areas, holothurians were the dominant megafaunal group accounting for 70.9 ± 26.3% and 67.7 ± 3.8 % of total megafaunal communities for 1ºN and 39ºN, respectively (Table 4). Due to the low quality of the video transects at 1ºN, it was not possible to estimate each holothurian species abundance with confidence. Concerning the holothurians sampled for this study, it was not possible to sample *D. validum* and

P. trachus at 39ºN and *S. globosa* at 1ºN, as they were absent along the dive transects (Table 2). Other megafauna that were observed in video images, but not sampled were Asteroidea, Actinaria, Gastropoda, Gorgonaria, Ophiuroidea, Echinoidea, Crinoidea and Pennatularia.

3.2 *Lipid compositions in holothurian tissue*

There were no qualitative variations in sterol and fatty acid composition between different sampling transects at the same station. Thus, data are presented separately for 1ºN and 39ºN as means with respective standard deviations for each site.

Among lipid compounds of holothurians (Table 5 a, b), sterols dominated the total extracted lipids at 1ºN, accounting for 84.25 ±2.00% in *D. validum*, 36.99 ± 16.74% in *P. longicauda* and 63.98 ± 28.60% in *P. trachus* of total lipids, whereas at 39ºN, they accounted for 48.23 ± 19.73% in *S. globosa* and 90.03 % of total lipids in *P. longicauda* (Table 5 a,b, Appendix S1,2 Table a-d). *n*-Alcohols contributed less than 1% to the total lipid pool. Differences between deep-sea holothurians in tissue samples from the 2 regions with respect to lipid classes were tested. 39ºN was significantly different in terms of lipid classes composition to 1ºN (ANOSIM, R=0.153, p=0.004).

At both 1ºN and 39ºN, FA ranged in carbon numbers from 14 to 25, with the dominant saturated FA being C_{14}, C_{16} and C_{18} (Appendix S1 and S2). MUFAs were dominated by the $C_{16:1}$, $C_{20:1}$, $C_{21:1,}$ $C_{23:1}$ and $C_{24:1}$ compounds. PUFA distributions were dominated by $C_{20:5}$ and $C_{20:4}$, which were the most abundant FA in all species. $C_{20:4}$ dominated 1ºN with 6.0 ± 1.5%, 8.6 ± 6.8% and 12.0 ± 8.7 % for *D. validum, P. longicauda* and *P. trachus* respectively, whereas at 39ºN, $C_{20:5}$ dominated the fatty acid profile with 9.5 ± 7.5% and 3.4% for *S. globosa* and for *P. longicauda* respectively (Appendix S1 and S2). As for the sterols, the most abundant included C_{27} Δ^7 and $C_{29}\Delta^0$ at both 1ºN and 39ºN, the latter being the most abundant.

Average values of the diagnostic lipid indices (Kiriakoulakis et al., 2011, Duineveld et al., 2012) in the holothurians tissues at 1ºN and 39ºN are shown in Figure 4. Bacteria-index FA dominated holothurian FA at 1ºN, particularly for *P. longicauda* and *P. trachus*. On the other hand, phytoplankton-index FA dominated holothurian FA at 39ºN, both for *S. globosa* and *P. longicauda* even though only one specimen was examined for *P. longicauda*. The MDS ordination plot did show a clear

355 separation between centroids corresponding to indices and regions (Figure 5).
356 Differences in these indices between the regions were tested with the PERMANOVA,
357 which demonstrated to be significantly different (MS=46.515, pseudo-F=8.809,
358 $p<0.001$, full details are reported in Appendix S3 a). The dissimilarity between the
359 regions was explained by a higher value of the phytoplankton lipid index in 39ºN,
360 while the bacterial lipid index explained the similarity between the holothurian tissues
361 (Appendix S3 b, 3c).
362
363 3.4 *Stable isotopic compositions and C/N ratios the holothurians*
364 Stable isotope ratios did not contradict the holothurian feeding habits
365 suggested by the FA compositions. At 1ºN, $\delta^{13}C$ and $\delta^{15}N$ values were -19.40 ± 0.27
366 ‰ and 12.10 ± 0.42 ‰ for sediments and -16.90 ± 0.81 ‰ and 16.37 ± 0.38 ‰ for
367 averaged holothurians, while at 39ºN, $\delta^{13}C$ and $\delta^{15}N$ values were -20.78 ± 0.11‰ and
368 6.84 ± 0.30 ‰ for sediments and -16.13 ± 0.25 ‰ and 10.50 ± 0.86 ‰ for
369 holothurians (Figure 6). For both regions, $\delta^{13}C$ values of sediment had lower values
370 than holothurians, by 2.5‰ in 1ºN and 4.7‰ at 39ºN. As for $\delta^{15}N$, holothurians
371 exhibited approximately 3.7‰ higher values than those of the sediments at both 1ºN
372 and 39ºN (Figure 6), which corresponds to approximately one trophic level
373 differences (Minagawa and Wada 1984). There was no substantial difference in $\delta^{15}N$
374 values between species collected at 1ºN, while *P. longicauda* had higher $\delta^{15}N$ values
375 than *S. globosa* by 1.5‰ at 39ºN. The C/N ratios of the holothurians did not differ
376 significantly between species and both areas (Chi-square= 13.545, $p<0.001$, full
377 details are reported in Appendix S3, Table d, Figure 7).
378
379 **4. Discussion**
380 In this study, we investigated the bioavailability of OM in deep-sea sediments
381 of two regions of contrasting primary production and discuss their effect on the lipid
382 composition of a range of abyssal holothurians.
383
384 *4.1. Sediment OM quantity and quality*
385 We first examined the labile portion of the OM in the sediment, as possible
386 energy sources for the holothurians, consisting of proteins, carbohydrates and lipids
387 (Danovaro et al. 2001; Amaro et al., 2010). For both regions, OM in the sediment is
388 composed mainly by carbohydrates, followed by proteins and lipids (Figure 3A). The

389 contribution of carbohydrates to the total biopolymeric C was higher at 1ºN than at
390 39ºN, while that of proteins and lipids was higher at 39ºN than at 1ºN (Figure 3B).
391 Although carbohydrate concentrations were higher than proteins at 39ºN, the
392 sedimentary protein concentrations were almost 2-fold higher than at other abyssal
393 plains (i.e. Porcupine Abyssal Plain-PAP) (Danovaro et al., 2001). In other studies,
394 the availability of proteins in deep-sea sediments was even lower than at the PAP
395 (Sibuet, 1984; Pfannkuche and Thiel, 1987; Danovaro et al., 1993; Boetius et al.,
396 1996; Tselepides et al., 2000), being in the same range as for the sediment in 1ºN.
397 These differences in sedimentary protein can be explained by the higher
398 concentrations of fresh OM supply, as indicated by phytopigment being 16-times
399 greater at 39ºN than in 1ºN (Figure 3A) and the presence of high concentrations of
400 carbohydrates. These high concentrations of labile OM in sediment is caused by high
401 OM flux to the seafloor and subsequent bioturbation of recently sedimented OM by
402 both macro-and-megafauna (Jumars et al., 1990), which were also abundant at 39ºN
403 (Table 3), although the burial process is not examined in this study. The significantly
404 higher C/N of sedimentary OM in 39ºN (Table 3) could be due to higher proportion of
405 carbohydrates, which are N-poor. This contradicts conventional wisdom (Meyers,
406 1994) with respect to OM quality and C/N, as typically higher values of the latter are
407 considered to be indicative of lower quality and this is not the case here. Although the
408 differences could also be due to sampling during different seasons (the sampling at
409 1ºN was during September and November and at 39ºN was during May and July),
410 NPP at the surface ocean at 39ºN is always higher than at 1ºN
411 (https://neo.sci.gsfc.nasa.gov/view.php?datasetId=MY1DMM_CHLORA), so the
412 sedimentary OM likely integrates these differences. We need further characterization
413 of the OM of deep-sea sediments to evaluate the relationships between C/N ratios and
414 OM quality.
415
416 *4.2. Holothurian food sources inferred from lipid compositions*
417 In this study we estimated very high abundances of abyssal holothurians at
418 39ºN (1433.8 ± 593.4 ind./ha), while at 1ºN the densities were low (40.2±27.4
419 ind./ha) (Table 4). OM quality followed the same gradient, in particular for
420 phytopigments (as discussed in section 4.1.). For comparison, at station M (Northeast
421 Pacific), holothurian densities were reported to increase from very low (0-19 ind./ha)
422 to very high numbers (11627 ind./ha) during a period of high food availability

(Huffard et al., 2016). Thus, we suggest that also here, high densities of abyssal holothurians at 39ºN is linked with the high quality of the sedimentary OM associated with the net primary production at the surface.

At the deep-sea floor, it might be expected that with changes in food source/quality, the biochemical composition of holothurians could change, for example in build-up of labile FA and other compounds at times of high (seasonal) supply. In the present study, the extracted lipids of all holothurian species were dominated by sterols. As for the FA, PUFAs and MUFAs comprise different and variable proportions between species. Lipid classes were significantly different between the two regions studied (see Table 5, Appendix S1 and 2, Table a-d). In marine environments, PUFAs are indicators of fresh labile OM and they are thought to mainly derive from phytoplankton (e.g., Parrish et al. 2000, Kiriakoulakis et al., 2007). Diatoms, which biosynthesise mostly $C_{20:5}$ (Volkman et al. 1989) and dinoflagellates, which produce more $C_{22:6}$ (Sargent et al. 1987; Harvey et al. 1988) are the main source of PUFAs in the phytoplankton. Here, concentrations of the main indices were significantly different in the two regions studied (Appendix S3 a), being phytoplankton FA index is responsible for these differences (Appendix S3 b). According to Kharlamenko et al. (2018), the relationship between the ratio of 20:5/20:4 and the trophic position of the organisms can be also informative for trophic studies as 20:5 is an index of fresh OM. In this study, all the holothurians analysed at 39ºN had a larger ratio (1.7 ± 1.6 for *S. globosa* and 7.2 for *P. longicauda*) than at 1ºN (0.3 ± 0.3 for *D. validum*, 0.2 ± 0.3 for *P. longicauda* and 0.03 ± 0.02 for *P. trachus*). This is in agreement with our previous conclusions that more labile OM supply at 39ºN is linked to high surface productivity.

Likewise, the presence of cholesta-, sitosta- and stigmasta-type (C_{27}, C_{28} and C_{29}), sterols together with 4□-methylcholestanol and its derivatives in *S. globosa* at 39ºN are also consistent with an origin from direct uptake of phytoplankton-derived OM via deposit-feeding on freshly deposited material (Santos et al., 1994, Hudson et al., 2004, Neto et al., 2006). The presence of a higher contribution of $C_{29}\Delta^0$ and $C_{29}\Delta^{5,22}$ in the muscle tissues of the holothurians (Table 5, Appendix S1, S2 c) reflects a phytoplankton community diet origin, whereas the contribution of $C_{27}\Delta^5$ (and other C_{27} sterols such as $C_{27}\Delta^{5,22}$ and $C_{27}\Delta^{22}$) and $C_{28}\Delta^{5,22}$ may reflect the dominance of invertebrate dietary sterols, though $C_{27}\Delta^5$ may also be originated from *de novo* synthesis by holothurians (Hernandez-Sanchez et al., 2010, 2012, Korb et al., 2010).

457 This suggests that the potential food source is different in the two areas, with
458 phytoplankton possibly being a more important food source for 39ºN than for 1ºN, as
459 it is indicated by the FA.

460 Zooplankton carcases (remains) may also be a potential food source for the
461 holothurians. The presence of alcohols or FA ($C_{20:1}$ and $C_{22:1}$) in holothurian tissues at
462 both regions (Appendix S1 and S2) suggests that zooplankton carcasses or moults,
463 and/or macrofauna, as a part of dietary source. Although we cannot be absolutely
464 confident that these compounds are biosynthesized by zooplankton and not by deep-
465 sea holothurians, their low concentrations in holothurians suggests that metazoan-
466 derived OM is not their primary food source.

467 There were differences in bacterial FA concentrations both between species
468 and regions. At 1ºN, the bacterial index FA of *D. validum* suggested small proportions
469 for this potential food source, whereas *P. longicauda* and *P. trachus* had a much
470 higher bacterial index than phytoplankton and zooplankton indices. At 39ºN, bacterial
471 FA accounted for 13.9 ± 2.0 % in *S. globosa*, where for *P. longicauda* bacterial FA
472 accounted for only 2.4%. The same result can be seen in Appendix S3c, inferring that
473 the bacterial index can be responsible for the differences between the regions.
474 Bacterial biomarkers are commonly found in suspended POM (e.g., Kiriakoulakis et
475 al. 2001), as well in surface sediments (e.g., Nomaki et al. 2009) and so their presence
476 in high amounts in the holothurian tissue is unsurprising. Amaro et al. (2012) found
477 that ca. 40% of bacterial OTUs were associated uniquely with the gut contents (i.e.,
478 absent in surrounding sediments) of *Molpadia musculus*, suggesting an occurrence of
479 wide and highly diversified interactions between prokaryotes and deep-sea
480 holothurians. *Psychropotes longicauda* was the species that showed the highest
481 percentage for bacterial FA at 1ºN. As this region is poor in fresh OM (Figure 4a),
482 this holothurian most likely feeds on relatively refractory OM in sediments and may
483 rely on microbial degradation and/or fermentation to break down the recalcitrant OM
484 as for *Molpadia blakei* at the PAP (Ginger et al., 2000). Nevertheless, it is not yet
485 clear whether these FA are dietary or symbiotic. Furthermore, the large differences in
486 relative abundances of bacterial FA between different species (Figure 4) infer that
487 they have different feeding habits or perhaps different association of microbial flora
488 in their gut. However, caution is needed for such an interpretation and it is
489 recommended to use these data qualitatively rather than quantitatively way estimating
490 the microbial contributions to the organic pools (Parrish et al. 2000, Kiriakoulakis et

al., 2005).

Due to logistical constraints, our comparative study between the regions covered only a short period, and sampling could not be conducted in the same season, nor at the same water depth. We were also unable to sample more holothurian species to have a better statistical comparison for both regions. In addition, we need to be careful in further interpreting our results, as biochemical responses of holothurians also appear to depend on their feeding mode and rate of locomotion (Neto et al., 2006). Highly mobile surface deposit-feeding holothurians quickly utilize fresher surface material, leaving larger, slower subsurface deposit feeders to consume more degraded forms (Iken et al., 2001). As a result, these holothurians gain competitive advantage over those that are restricted by slower locomotion, non-selective feeding tentacle morphology and/or physiological limitations (Hudson et al., 2003; Iken et al., 2001; Neto et al., 2006; Wigham et al., 2003). For example, *S. globosa* is a mobile (Lafond, 1967; A. Smith et al., 1997) elasipodid holothurian, which results in a rapid exploitation of horizontal patchiness in recently deposited, food-rich particles being suggested to feed selectively (Miller et al., 2000). In contrast, *P. longicauda* and *P. trachus* are big and not very mobile, feeding on more refractory material. These behaviors may be reflected in the FA compositions, as phytoplankton FA proportions are higher in *S. globosa* than at *P. longicauda* and *P. trachus* (Figure 4). Furthermore, it would be expected to link seasonal input of fresh OM to a change in their body composition by building-up of labile FA during the fresh material bloom. Thus, we can suggest that this differential access to food leads to interspecific differences in reproductive effort and ultimately abundance (Ramirez-Llodra et al., 2005), which displays differences in their FA composition (Hudson et al., 2004). Some species display a high degree of seasonal bentho-pelagic coupling, while others have different fatty acids composition that may be related to a different reproduction behavior. Caution is recommended to further interpretations.

4.3. Stable isotopic compositions of holothurians and sediments

For both regions, the $\delta^{13}C$ enrichments between surface sediment (possible food source) and holothurians were larger than 1 ‰. Isotopic fractionations of $\delta^{13}C$ are sometimes diverse (McCutchan et al. 2003), so the large differences in this study may be also partly due to the variations that exist within species-specific or feeding-specific habit (Vander Zanden and Rasmussen 2001). Selective ingestion or digestion

525 of OM within sediment may also contribute to such high variability, as phytodetritus
526 or bacteria can have different $\delta^{13}C$ values from the bulk of TOC. The same
527 phenomena have already been reported in other studied areas (Michel et al., 2016,
528 Mincks et al. 2008).

529 The higher $\delta^{15}N$ values of holothurians at 1ºN *vs.* 39ºN may reflect the
530 differences in that of surface sediments (Figure 6). The amplitude of $\delta^{15}N$-enrichment
531 in the holothurian tissue corresponds to *ca.* 1 trophic level (3 to 4‰) or slightly larger
532 from surface sediments, suggesting that, in both regions, holothurians primarily ingest
533 and digest these materials. Holothurian $\delta^{15}N$ values did not differ between taxonomic
534 groups in either region, indicating that these organisms share the same trophic level
535 without any niche separation between taxa. At both 1ºN and 39ºN, the slightly larger
536 enrichment in $\delta^{15}N$ (3 to 4‰) could reflect some degree of microbial-mediated OM
537 degradation. Whilst, studies have shown that some holothurians preferentially feed on
538 fresh OM (Billett, 1991, Smith et al., 1999, Smith et al., 2008, Amaro et al., 2010),
539 there is also evidence that they can feed on bacteria (Amaro et al., 2012) or on both
540 (Sibuet et al., 1982, Amaro et al., 2010). This assumption does not contradict the lipid
541 data presented here, which showed substantial contribution of bacterial FA in some
542 holothurian tissues (Figure 5).

543 There were no significant differences in C/N ratios between the holothurians'
544 tissue when comparing the regions (Figure 7, Appendix S3, Table d). The C:N ratios
545 for the holothurians are in range for most marine invertebrates (Mincks et al., 2008,
546 Nomaki et al., 2008) being often species-specific and regulated by a species
547 physiology (Raubenheimer et al., 2004).

548 Despite the intrinsic limitations and constraints due to the difficulty in
549 working at abyssal depths, the results reported here suggest that changes in upper
550 ocean productivity, altering the quality and quantity of OM reaching the deep-sea
551 floor is associated with the high abundances of the megabenthos and a different lipid
552 composition. The variations in FA and sterol compositions between species and
553 regions may be linked to their feeding habits. However, processes other than just the
554 availability of fresh OM regulate the proportions of fatty acids in the tissues of deep-
555 sea holothurians and some caution is needed on the interpretation of the data.
556 Although more work on how biochemical needs of deep-sea holothurians determine
557 dietary needs and how this changes temporally with OM quality and quantity; this
558 study improves our understanding into the ecosystem functioning in abyssal plains.

559

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Legends to Figures

Figure 1. Bathymetry map of the 2 different locations (1ºN-01º15'N, 163º15'E, 4277-m depth; 39ºN-39º00' N, 146º00'E, 5260-m depth) in the Western North Pacific (Table 1).

Figure 2. Seafloor images of both regions located at the western North Pacific: a, and b represent 1ºN; c and d represent 39ºN.

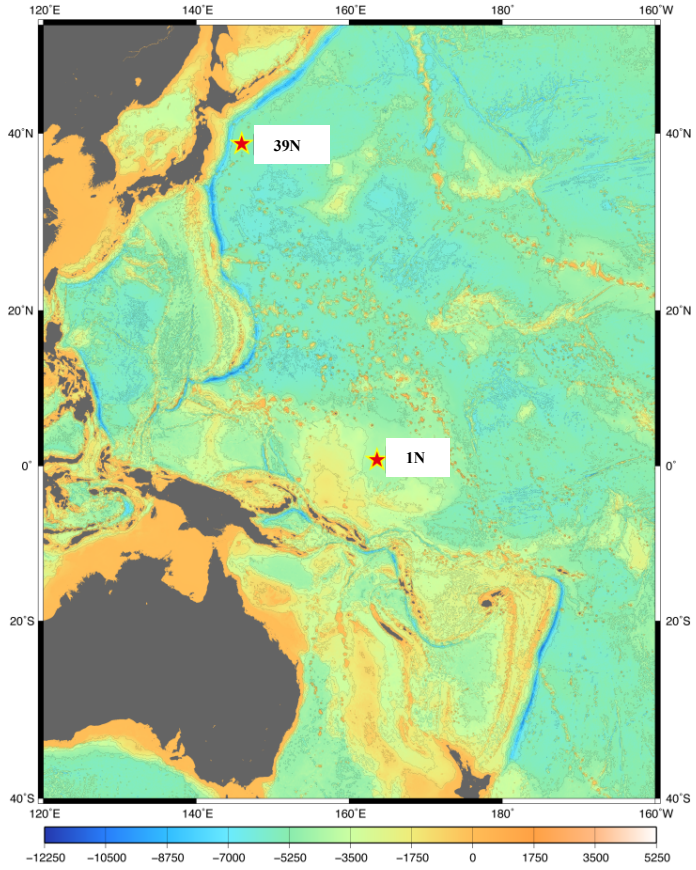
Figure 3. Concentration of A) total phytopigments, carbohydrates, proteins and lipids and B) % contributions of biopolymeric carbon in the surface sediments.

Figure 4. Diagnostic of the lipid indices in the holothurians tissue at A) 1ºN and B) 39ºN.
See Table a-f, Appendix I, II and Material and Methods for explanation of indices.

Figure 5. MDS plot of lipid indices (phytoplankton, zooplankton and bacterial fatty acids) in the holothurians tissue at 1ºN (A) and 39ºN (B).

Figure 6. Carbon and nitrogen isotopic compositions of surface sediments and holothurians. Reported are averaged values for the sediment and holothurians tissue at both stations (1ºN, 39ºN).

Figure 7. Average C/N ratios from the first 1 cm depth of sediment and holothurians tissue at both stations (1ºN, 39ºN).


120°E
140°E
160°E
180°
160°W
40°N
20°N
0°
20°S
40°S
39N
1N
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−10500
−8750
−7000
−5250
−3500
−1750
0
1750
3500
5250

Figure 1

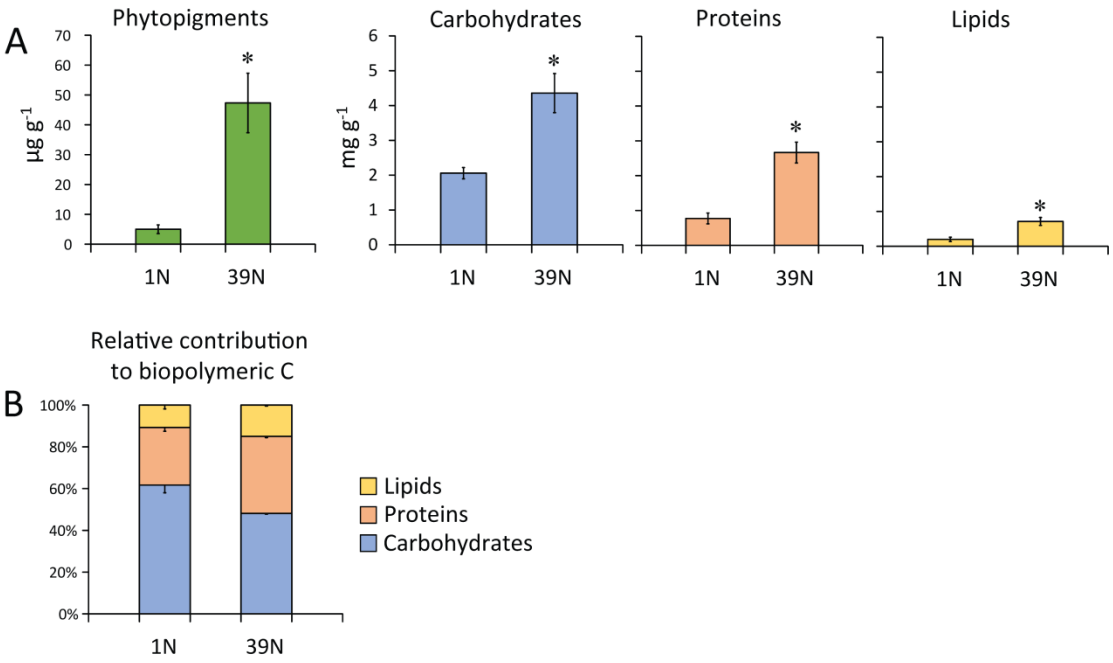
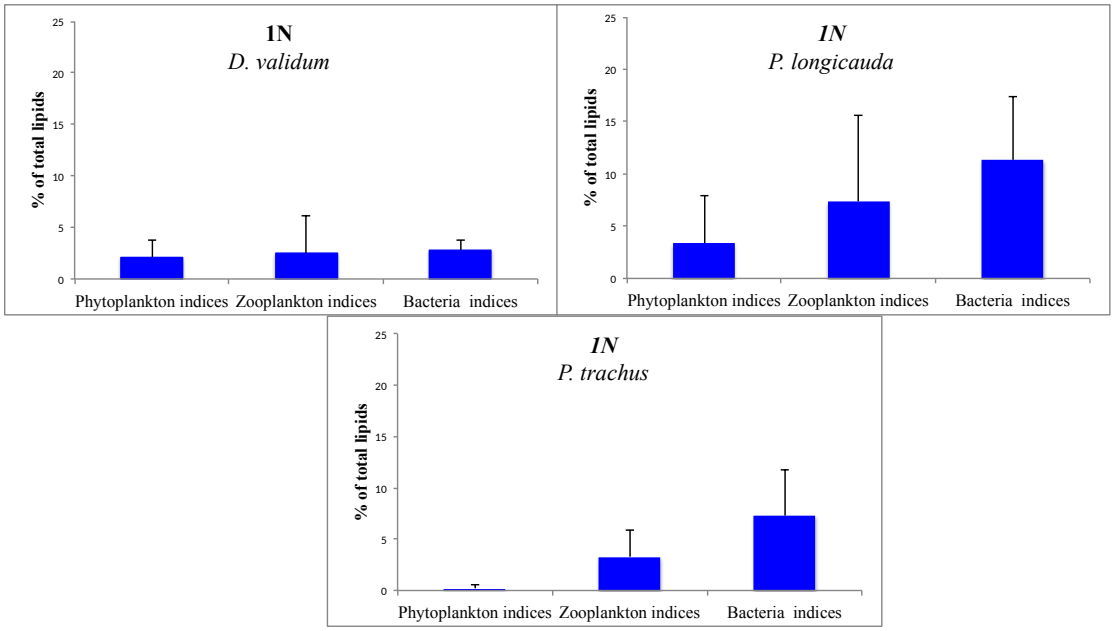

A
Phytopigments
µg g-1
70
60
50
40
30
20
10
0
1N
39N
Carbohydrates
mg g-1
6
5
4
3
2
1
0
1N
39N
Proteins
1N
39N
Lipids
1N
39N
B
Relative contribution to biopolymeric C
100%
80%
60%
40%
20%
0%
1N
39N
Lipids
Proteins
Carbohydrates

Figure 3

A)


1N
D. validum
% of total lipids
Phytoplankton indices
Zooplankton indices
Bacteria indices
1N
P. longicauda
% of total lipids
Phytoplankton indices
Zooplankton indices
Bacteria indices
1N
P. trachus
% of total lipids
Phytoplankton indices
Zooplankton indices
Bacteria indices

B)

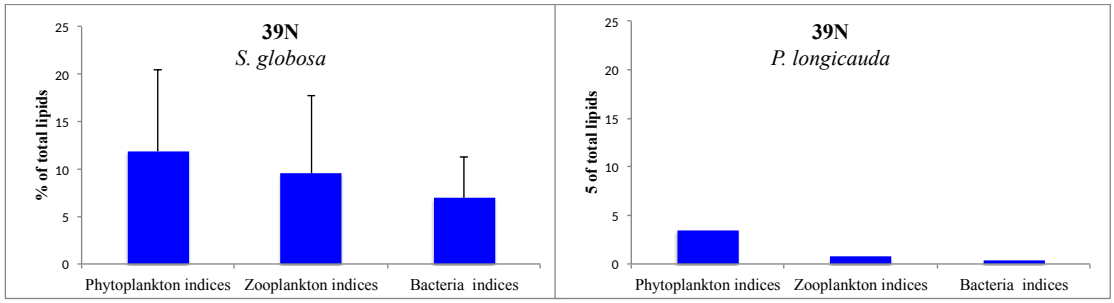

39N
S. globosa
% of total lipids
Phytoplankton indices
Zooplankton indices
Bacteria indices
39N
P. longicauda
5 of total lipids
Phytoplankton indices
Zooplankton indices
Bacteria indices

Figure 4

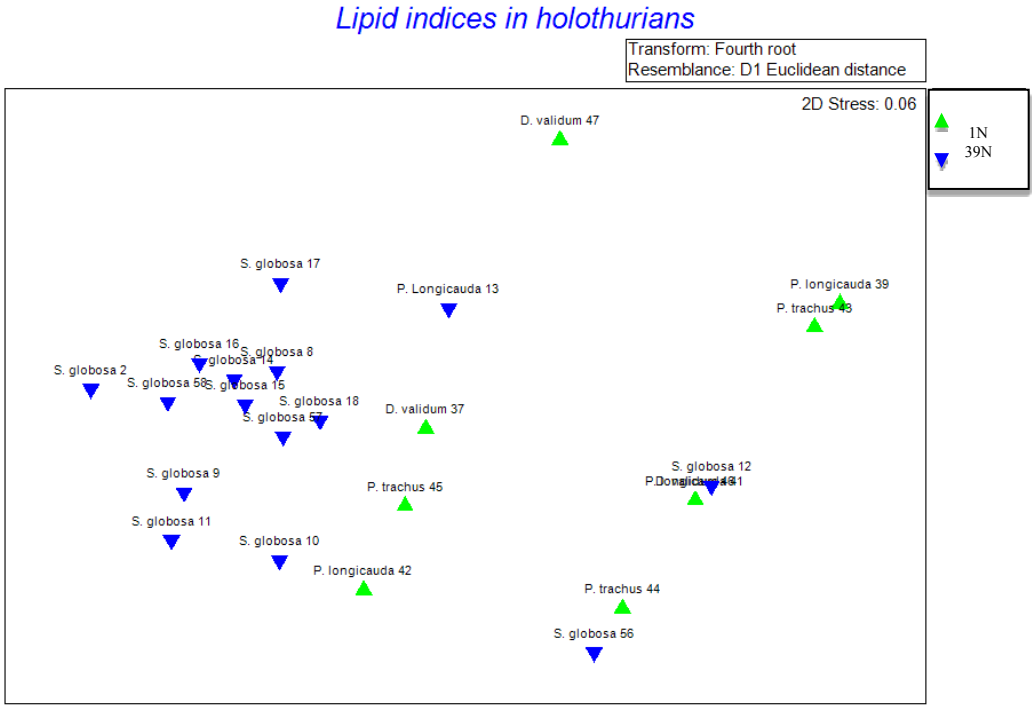

Lipid indices in holothurians
Transform: Fourth root
Resemblance: D1 Euclidean distance
2D Stress: 0.06
1N
39N
D. validum 47
S. globosa 17
P. Longicauda 13
P. longicauda 39
P. trachus 49
S. globosa 16
S. globosa 8
S. globosa 14
S. globosa 2
S. globosa 58
S. globosa 15
S. globosa 18
D. validum 37
S. globosa 57
S. globosa 9
P. trachus 45
S. globosa 12
S. globosa 11
S. globosa 10
P. longicauda 42
P. trachus 44
S. globosa 56

Figure 5

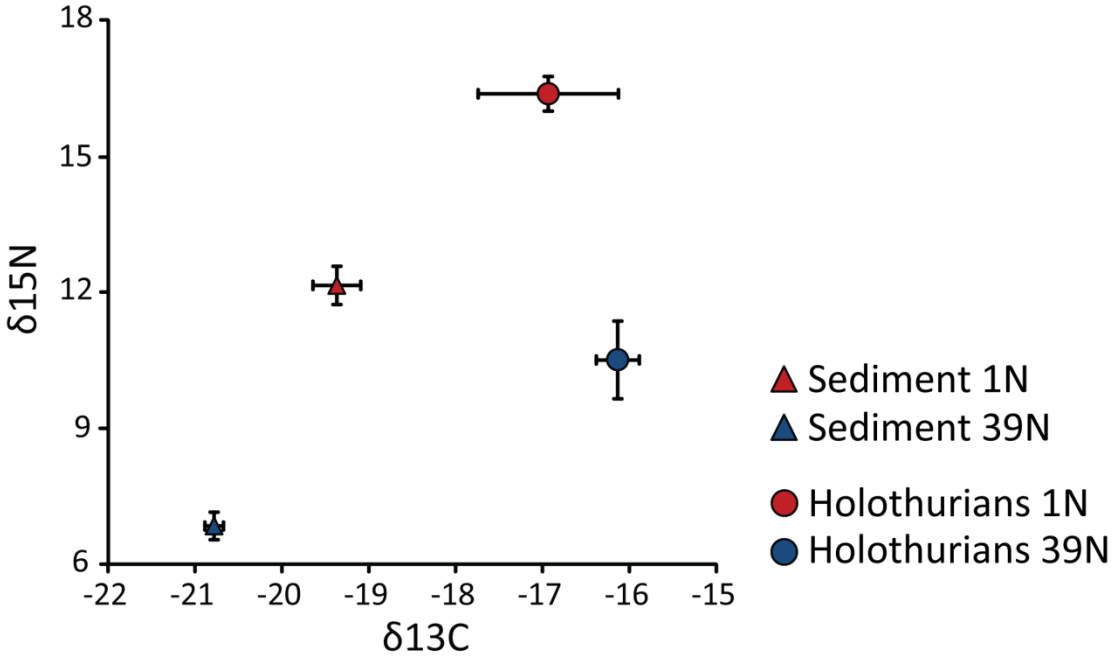

18
15
12
9
6
δ15N
-22
-21
-20
-19
-18
-17
-16
-15
δ13C
Sediment 1N
Sediment 39N
Holothurians 1N
Holothurians 39N

Figure 6

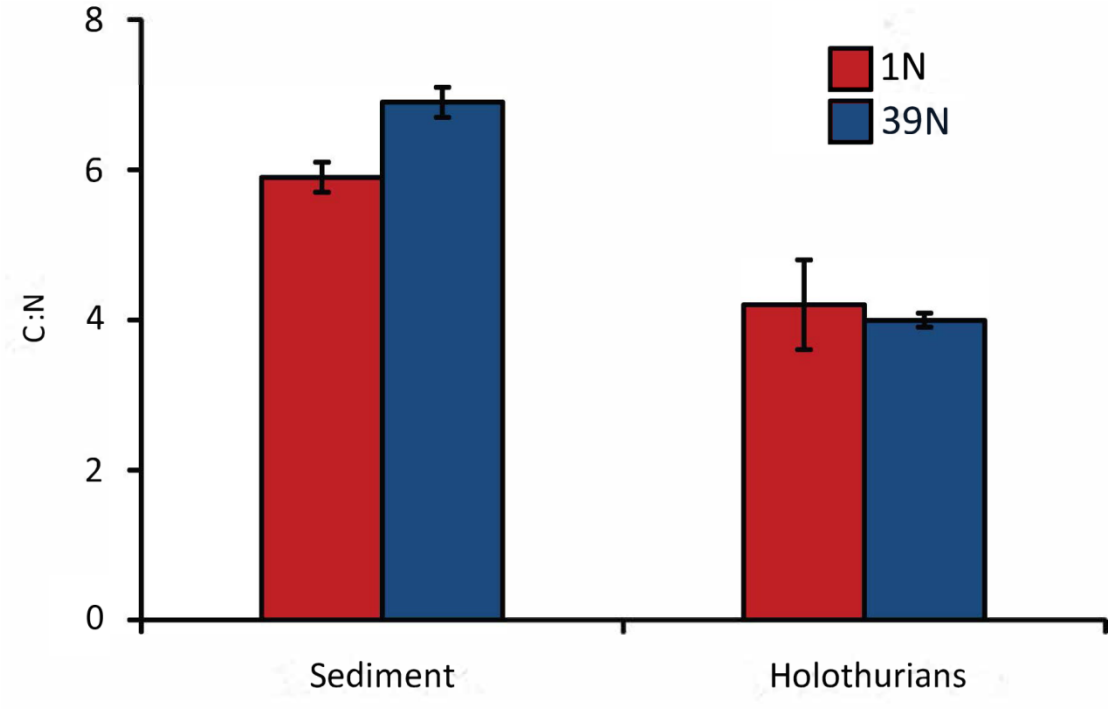


Figure 7

Table 1 – Details of the five independent transects made for each location studied in the western Pacific Ocean.

Location	Dive	Cruise	Latitude (start)	Longitude (start)	Latitude (end)	Longitude end)	Area analised (ha)	Depth range (m)
1ºN	1367	YK1309	1º15'07"N	163º14'87"E	1º14'99"N	163º14'75"E	0.221	4277
	1368	Y1309	1º15'08"N	163º14'75"E	1º15'02"N	163º14'96"E	0.176	4277
	1375	Y1312	1º15'07"N	163º14'69"E	1º14'88"N	163º14'99"E	0.493	4278
39ºN	1395	YK1412	39º0'08"N	146º0'40"E	38º59'97"N	146º0'22"E	0.272	5260
	1396	YK1412	39º0'10"N	146º0'22"E	38º59'63"N	146º0'04"E	0.345	5260

Table 2 – Holothurian samples collected at the 1ºN and 39ºN in the western Pacific Ocean.

Location	Species	Order	Number
1ºN	*Deima validum*	Elasipodida	3
	Psychropotes longicauda	Elasipodida	3
	Pseudostichopus trachus	Aspidochirotida	3
39ºN	*Scotoplanes globosa*	Elasipodida	14
	Psychropotes longicauda	Elasipodida	1

Table 3 – Mean total organic carbon (TOC), mean total nitrogen (TN) as % in surface sediments (0-5 cm) and mean molar C/N for both 1ºN and 39ºN.

	TOC	TN	C/N
1ºN	1.05 (0.19)	0.18 (0.03)	5.87 (0.23)
39ºN	1.28 (0.11)	0.18 (0.02)	6.98 (0.15)

Table 4 – Total megafauna and holothurian abundances for each transect made at each location studied. Average % of holothurians is also represented.

Location	Dive	Cruise	Average_megafauna	Average_holothurians	Average_% holothurians
1ºN	1367	YK1309	54.5 (27.4)	40.2 (27.4)	70.9 (26.3)
	1368	YK1309			
	1375	YK1312			
39ºN	1395	YK1412	2144.4 (593.4)	1433.8 (593.4)	67.7 (3.8)
	1396	YK1412			

Units – Individuals/ha; standard deviation in parentheses.

Table 5 – Mean total concentrations of lipids for holothurians from a) 1ºN and b) 39ºN.

a)

	MUFAs	PUFAs	Sat._FAMES	Sterols	Alcohol
D. validum	198.93 (125.99)	864.20 (494.41)	98.16 (40.72)	6468.39 (2435.17)	6.92 (8.08)
P. longicauda	2832.47 (3497.95)	2048.53 (1492.87)	1268.67 (1012.55)	5344.43 (6634.55)	39.96 (43.59)
P. trachus	500.92 (747.22)	1100.50 (1577.81)	680.19 (942.80)	3033.80 (2628.29)	2.01 (1.74)

Units - µg. g^{-1} of dry tissue; n = 3, standard deviation in parentheses.

b)

	MUFAs	PUFAs	Sat._FAMES	Sterols	Alcohol
S. globosa	2022.41 (1557.57)	1910.53 (960.32)	530.65 (323.51)	4641.98 (2289.80)	36.45 (34.32)
P. longicauda	31.60	39.21	21.56	887.00	5.87

Units - µg. g^{-1} of dry tissue; n = 14, standard deviation in parentheses for *S. globosa*, but n=1 for *P. longicauda*.

Appendix S1

Table a - Total unsaturated FAMES of dry tissue for holothurians from 1ºN. Results are presented as mean % ± Stdev of the total lipids; n=3.

Compound	*D. validum*	*P. longicauda*	*P. trachus*
$C_{14:1}$	0.00 (0.00)	0.00 (0.00)	0.07 (0.12)
$C_{16:2}$	0.01 (0.01)	0.00 (0.00)	0.62 (1.08)
$C_{16:1}$	0.03 (0.02)	2.14 (2.81)	1.73 (2.41)
$C_{17:1}$	0.01 (0.01)	5.50 (7.23)	0.19 (0.33)
$C_{18:2}$	0.01 (0.01)	0.00 (0.00)	0.28 (0.47)
$C_{18:1}$	0.14 (0.13)	1.12 (0.38)	1.64 (1.42)
$C_{19:2}$	0.00 (0.00)	0.00 (0.00)	0.03 (0.05)
$C_{19:1}$	0.09 (0.13)	0.00 (0.00)	0.16 (0.28)
$C_{20:6}$	0.00 (0.00)	0.33 (0.58)	0.00 (0.00)
$C_{20:5}$	2.09 (1.73)	3.15 (4.44)	0.22 (0.37)
$C_{20:4}$	5.96 (1.45)	8.65 (6.82)	12.01 (8.72)
$C_{20:3}$	0.09 (0.16)	2.75 (3.72)	0.09 (0.15)
$C_{20:2}$	0.12 (0.13)	1.97 (2.88)	0.50 (0.55)
$C_{20:1}$	2.12 (3.41)	5.42 (8.34)	1.01 (1.25)
$C_{21:4}$	1.41 (0.67)	0.34 (0.58)	2.39 (1.51)
$C_{21:1}$	0.02 (0.03)	3.42 (5.92)	0.15 (0.26)
$C_{22:6}$	0.00 (0.00)	0.21 (0.19)	0.00 (0.00)
$C_{22:5}$	0.00 (0.00)	0.05 (0.09)	0.00 (0.00)
$C_{22:4}$	0.89 (0.79)	1.63 (0.15)	1.90 (2.71)
$C_{22:3}$	0.03 (0.05)	0.00 (0.00)	0.00 (0.00)
$C_{22:2}$	0.00 (0.00)	0.05 (0.09)	0.00 (0.00)
$C_{22:1}$	0.13 (0.09)	0.92 (0.86)	1.34 (1.26)
$C_{23:4}$	0.00 (0.00)	0.06 (0.10)	0.00 (0.00)
$C_{23:2}$	0.00 (0.00)	0.92 (1.60)	0.00 (0.00)
$C_{23:1}$	0.74 (0.27)	0.68 (0.63)	2.01 (2.33)
$C_{24:2}$	0.00 (0.00)	0.21 (0.36)	0.00 (0.00)
$C_{24:1}$	0.11 (0.12)	0.31 (0.44)	0.77 (0.66)
co eluting $C_{25:1}$ and C_{25}	0.02(0.04)	0.09 (0.16)	0.00 (0.00)
Unknown	0.00 (0.00)	1.31 (1.14)	0.00 (0.00)

Table b - Total saturated and branched (iso/anteiso) FAMES of dry tissue for holothurians from 1ºN. Results are presented as mean % ± Stdev of the total lipids; n=3.

Compound	*D. validum*	*P. longicauda*	*P. trachus*
$C_{14:0\ i}$	0.00 (0.00)	0.13 (0.21)	0.15 (0.06)
$C_{14:0\ a}$	0.00 (0.01)	0.00 (0.00)	0.00 (0.00)
$C_{14:0}$	0.00 (0.01)	4.48 (5.94)	0.14 (0.19)
$C_{15:0\ i}$	0.02 (0.01)	0.03 (0.05)	0.35 (0.13)
$C_{15:0\ a}$	0.05 (0.02)	0.18 (0.18)	0.68 (0.92)
$C_{15:0}$	0.00 (0.02)	1.02 (1.74)	0.29 (0.46)
$C_{16:0\ i}$	0.12 (0.14)	0.47 (0.48)	0.52 (0.74)
$C_{16:0\ a}$	0.02 (0.04)	0.00 (0.00)	0.03 (0.05)
$C_{16:0}$	0.04 (0.03)	1.96 (1.33)	1.33 (0.82)
$C_{17:0\ i}$	0.01 (0.02)	0.08 (0.07)	0.27 (0.25)
$C_{17:0\ a}$	0.04 (0.03)	0.03 (0.05)	0.04 (0.07)
$C_{17:0}$	0.09 (0.07)	1.36 (1.96)	0.42 (0.59)
$C_{18:0\ i}$	0.02 (0.01)	0.00 (0.00)	0.06 (0.10)
$C_{18:0}$	0.52 (0.62)	3.24 (4.24)	4.04 (6.28)
$C_{19:0}$	0.13 (0.09)	0.35 (0.51)	0.34 (0.31)
$C_{20:0}$	0.16 (0.15)	0.27 (0.34)	0.09 (0.16)
$C_{21:0}$	0.17 (0.26)	0.43 (0.73)	0.00 (0.00)
$C_{22:0}$	0.08 (0.12)	0.01 (0.17)	0.00 (0.00)
$C_{23:0}$	0.01 (0.01)	6.94 (12.02)	0.00 (0.00)
$C_{24:0}$	0.12 (0.16)	0.00 (0.00)	0.02 (0.03)
Unknown	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

Table c – Total mean sterol percentages of dry tissue for holothurians from 1ºN. Results are presented as mean % ± Stdev of the total lipids; n=3.

Compound	*D. validum*	*P. longicauda*	*P. trachus*
$C_{27}\,\Delta^{5,22}$	0.06 (0.10)	0.57 (0.57)	0.00 (0.00)
$C_{27}\,\Delta^{22}$	0.70 (0.20)	0.23 (0.20)	0.00 (0.00)
$C_{27}\,\Delta^{5}$	1.95 (1.37)	1.41 (0.38)	5.16 (3.98)
$C_{27}\,\Delta^{5,24}$	1.04 (0.55)	1.05 (0.93)	3.01 (4.26)
$C_{27}\,\Delta^{7}$	10.49 (14.11)	3.00 (0.93)	1.96 (2.77)
$C_{27}\,\Delta^{0}$	4.70 (1.19)	0.59 (0.75)	1.40 (0.23)
$C_{28}\,\Delta^{22}$	0.88 (0.79)	1.19 (1.14)	2.40 (3.40)
$C_{28}\,\Delta^{5,22}$	0.80 (1.38)	0.16 (0.28)	0.00 (0.00)
$C_{28}\,\Delta^{5,24}$	0.67 (0.90)	0.00 (0.00)	0.12 (0.18)
$C_{28}\,\Delta^{5}$	2.47 (2.34)	0.57 (0.99)	10.44 (12.64)
$4MeC_{28}\,\Delta^{0}$	0.62 (0.16)	0.14 (0.24)	0.40 (0.57)
$C_{29}\,\Delta^{5}$	2.14 (2.09)	0.63 (0.55)	2.03 (2.87)
$C_{29}\,\Delta^{7}$	12.26 (5.16)	3.11 (1.59)	7.70 (1.52)
$C_{29}\,\Delta^{0}$	45.47 (8.72)	24.33 (13.26)	44.62 (16.20)

Table d - Total alcohol percentages of dry tissue for holothurians from 1ºN. Results are presented as mean % ± Stdev of the total lipids; n=3.

Compound	*D. validum*	*P. longicauda*	*P. trachus*
$C_{16:0}$	0.03 (0.03)	0.65 (0.92)	0.12 (0.16)
$C_{18:0}$	0.03 (0.04)	0.07 (0.10)	0.06 (0.11)
$C_{20:0}$	0.04 (0.08)	0.00 (0.00)	0.00 (0.00)
$C_{24:0}$	0.03 (0.05)	0.00 (0.00)	0.00 (0.00)

Appendix S2

Table a - Total unsaturated FAMES of dry tissue for holothurians from 39ºN. Results are presented as mean % ± Stdev of the total lipids; n=14 for *S. globosa* and n=1 for *P. longicauda.*

Compound	*S. globosa*	*P. longicauda*
$C_{14:1}$	0.12 (0.39)	0.00
$C_{15:2}$	0.01 (0.02)	0.00
$C_{15:1}$	0.02 (0.04)	0.00
$C_{16:4}$	0.05 (0.17)	0.00
$C_{16:3}$	0.28 (1.05)	0.00
$C_{16:2}$	0.32 (0.83)	0.00
$C_{16:1}$	4.31 (4.76)	0.38
$C_{17:2}$	0.00 (0.01)	0.01
$C_{17:1}$	0.18 (0.33)	0.00
$C_{18:4}$	0.10 (0.29)	0.00
$C_{18:3}$	0.03 (0.07)	0.00
$C_{18:2}$	0.35 (0.53)	0.04
$C_{18:1}$	2.65 (2.24)	1.66
branched $C_{19:0}$?+mufa	0.05 (0.14)	0.00
$C_{19:5}$	0.01 (0.05)	0.00
$C_{19:4}$	0.00 (0.01)	0.00
$C_{19:2}$	0.34 (1.27)	0.00
$C_{19:1}$	0.12 (0.20)	0.00
$C_{20:5}$	9.53 (7.48)	3.45
$C_{20:4}$	5.37 (3.70)	0.48
$C_{20:3}$	0.48 (1.50)	0.00
$C_{20:2}$	0.39 (0.24)	0.00
$C_{20:1}$	5.22 (9.58)	0.00
$C_{21:5}$	0.06 (0.13)	0.00
$C_{21:4}$	0.64 (0.61)	0.00
$C_{21:1}$	2.40 (4.44)	0.06
$C_{22:6}$	2.36 (2.80)	0.00
$C_{22:5}$	0.80 (1.46)	0.00
$C_{22:4}$	0.49 (1.83)	0.00
$C_{22:3}$	0.39 (1.46)	0.00
$C_{22:2}$	0.00 (0.00)	0.00
$C_{22:1}$	2.87 (1.94)	0.48
$C_{23:5}$	0.00 (0.01)	0.00
$C_{23:1}$	1.26 (0.73)	0.33
$C_{24:2}$	0.53 (0.82)	0.00
$C_{24:1}$	1.49 (1.80)	0.28
co eluting $C_{25:1}$ and C_{25}	0.03 (0.07)	0.00
Unknown	2.03 (4.29)	0.00

Table b - Total saturated and branched (iso/anteiso) FAMES of dry tissue for holothurians from 39ºN. Results are presented as mean % ± Stdev of the total lipids; n=14 for *S. globosa* and n=1 for *P. longicauda*.

Compound	*S. globosa*	*P. longicauda*
$C_{14:0\ i}$	0.01 (0.29)	0.00
$C_{14:0\ a}$	0.03 (0.11)	0.00
$C_{14:0}$	0.46 (0.55)	0.03
$C_{15:0\ i}$	0.14 (0.15)	0.00
$C_{15:0\ a}$	0.12 (0.17)	0.00
$C_{15:0}$	0.43 (0.48)	0.05
$C_{16:0\ i}$	0.29 (0.56)	0.00
$C_{16:0\ a}$	0.22 (0.80)	0.00
$C_{16:0}$	1.69 (2.07)	1.25
$C_{17:0\ i}$	0.03 (0.07)	0.00
$C_{17:0\ a}$	0.19 (0.58)	0.00
$C_{17:0}$	0.31 (0.35)	0.06
$C_{18:0\ i}$	0.01 (0.01)	0.00
$C_{18:0}$	0.62 (0.43)	0.62
$C_{19:0}$	0.45 (1.08)	0.06
$C_{20:0}$	0.30 (0.17)	0.02
$C_{21:0}$	0.13 (0.38)	0.10
$C_{22:0}$	0.22 (0.58)	0.00
$C_{23:0}$	0.01 (0.02)	0.00
$C_{24:0}$	0.06 (0.14)	0.00
Unknown	0.15 (0.46)	0.00

Table c – Total sterol composition of dry tissue for holothurians from 39ºN. Results are presented as mean % ± Stdev of the total lipids; n=14 for *S. globosa* and n=1 for *P. longicauda.*

Compound	*S. globosa*	*P. longicauda*
$C_{27}\Delta^{5,22}$	0.72 (1.68)	1.23
$C_{27}\Delta^{22}$	1.55 (1.91)	3.24
$C_{27}\Delta^{5}$	4.16 (2.55)	9.93
$C_{27}\Delta^{5,24}$	1.87 (2.36)	2.22
$C_{27}\Delta^{7}$	4.56 (7.67)	0.00
$C_{27}\Delta^{0}$	2.88 (1.59)	6.05
$C_{28}\Delta^{22}$	1.70 (1.42)	16.91
$C_{28}\Delta^{5,22}$	1.95 (1.46)	3.87
$C_{28}\Delta^{5,24}$	1.98 (1.48)	0.00
$C_{28}\Delta^{5}$	2.03 (1.79)	2.96
$4MeC_{28}\Delta^{0}$	1.58 (3.11)	8.01
$C_{28}\Delta^{0}$	0.18 (0.69)	0.00
$C_{29}\Delta^{5}$	1.77 (3.15)	0.00
$C_{29}\Delta^{7}$	1.91 (0.96)	15.29
$C_{29}\Delta^{0}$	19.23 (7.82)	20.31
$C_{29}\Delta^{5,22}$	0.16 (0.32)	0.00

Table d - Total alcohol of dry tissue for holothurians from 39ºN. Results are presented as mean %± Stdev of the total lipids; n=14 for *S. globosa* and n=1 for *P. longicauda.*

Compound	*S. globosa*	*P. longicauda*
$C_{16:0}$	0.25 (0.37)	0.33
$C_{18:0}$	0.23 (0.34)	0.23
$C_{19:0}$	0.00 (0.01)	0.00
$C_{20:0}$	0.02 (0.04)	0.03

Appendix S3

Table a - Tests carried out to ascertain differences in the concentrations of the main indices (phytoplankton, zooplankton and bacterial fatty acids) in the two regions studied; DF = degrees of freedom, SS = sum of squares; MS = mean square; F = F statistic; P = probability level; *** = P <0.001; ns = not significant; na = not applicable.

PERMANOVA

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Indices /stations	1	46.515	46.515	8.8087	***	999
Res	22	116.17	5.2806			
Total	23	162.69				

Table b - SIMPER analyses of the dissimilarity between the stations with respect to diagnostic lipid indices. The % contribution by each index to the dissimilarity is listed in the last column.

Lipid index	Contribution %
Phytoplankton	40.62
Zooplankton	29.97
Bacteria	29.41

Table c- SIMPER analyses of the similarity in 1ºN (a) and 39ºN (b) with respect to diagnostic lipid indices. The % contribution by each index to the dissimilarity is listed in the last column.

a)

Lipid index	Contribution %
Bacteria	74.79
Zooplankton	19.88

b)

Lipid index	Contribution %
Bacteria	41.87
Phytoplankton	29.28
Zooplankton	28.85

Table d - Results of the Kruskal-Wallis ANOVA (a) tests carried out to ascertain differences in the C:N of sediment and in the tissue of the holothurians: dF =degrees of freedom, P = probability level, *** = P <0.001, ns = not significant.

	Chi-Square	df	Asymp. Sig.
sediment	13.545	1	***
Holothurian	0.007	1	n.s